

## SHORT COMMUNICATION

## Beringia, the Phylogeographic Origin of a Circumpolar Plant, *Vaccinium uliginosum*, in the Japanese Archipelago

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The intraspecific genetic diversity of the circumpolar *Vaccinium uliginosum* (Ericaceae) was examined to determine the origin of circumpolar plants that migrated to the Japanese archipelago during the cold periods of the Pleistocene. The high mountains of the Japanese archipelago are among the southernmost limits of distribution of circumpolar plant species. Two noncoding regions of the chloroplast DNA (*trnL-trnF* and *trnS-trnG* intergenic spacers) were sequenced to determine the phylogeographic histories of the plants inhabiting five sites in the Japanese mountains and one site on the Kamchatka Peninsula. The sequences were used to extend a previously constructed dataset on global phylogeography, which identified three main lineages: an arctic-alpine lineage, an amphi-Atlantic lineage, and a Beringian lineage. All three cpDNA haplotypes in the mountains of Japan and on the Kamchatka Peninsula belonged to the Beringian lineage. A key finding in this study is that Beringia appears to be the phylogeographic origin of *V. uliginosum* in the Japanese archipelago. The Beringian refugium hypothesis proposed by Eric Hulén may therefore be applicable to a significant portion of the Japanese alpine flora.

Key words: Beringia, chloroplast DNA, circum polar element, phylogeography, refugia

Circumpolar plants, as the name implies, occur around the far northern parts of the Northern Hemisphere and extend southward into the mountains of Europe, North America, and Asia. The Japanese archipelago is one of the southernmost limits of the ranges of several circumpolar plant species (e.g. *Dryas octopetala* L., *Diapensia lapponica* L., and *Vaccinium uliginosum* L.), all of which inhabit high mountains. The alpine flora of Japan shows a strong association with the circumarctic region (Koidzumi 1919, Kawano 1971, Toyokuni 1981, Shimizu 1982, 1983). In fact, more than a quarter (26.7%) of the species of alpine plants in Japan are circumpolar in their distribution (Shimizu 1982, 1983). Researchers have therefore considered significant parts of the Japanese alpine flora to be relics of circumpolar species that colonized the archipelago during cold

periods in the Pleistocene (Koidzumi 1919, Kawano 1971, Toyokuni 1981, Shimizu 1982, 1983).

During the few last decades, this hypothesis gained support from phylogeographic studies of Japanese alpine plants (reviewed in Fujii & Senni 2006; see also Fujii *et al.* 1997, 1999, Ikeda *et al.* 2006, 2009a, 2009b). Studies of several unrelated alpine species have revealed clear genetic differences between populations in central Japan and those in northern Japan, suggesting that alpine plants migrated to the Japanese archipelago at least twice during the Quaternary climate cycles. The origins of the circumpolar plants in Japan were not discussed, however, because those studies did not include samples from more northerly regions. Although previous studies revealed the global phylogeography of some circumpolar spe-

cies (e.g., Abbott *et al.* 2000, Abbott & Comes 2003, Schönswetter *et al.* 2006, Ehrich *et al.* 2008), they did not include samples from the Japanese alpine zone, even though Japan is among the southernmost areas where circumpolar plants occur (e.g., Skred *et al.* 2006). To determine the origin of Japanese alpine plants and the global phylogeography of circumpolar plants, we examined the intraspecific genetic diversity of *Vaccinium uliginosum* L. s. lat. (Ericaceae) in the Japanese archipelago and in neighboring regions. Investigations of the global phylogeography of *V. uliginosum* based on chloroplast DNA (cpDNA) identified the following three major lineages: the Arctic-Alpine lineage, the Amphi-Atlantic lineage, and the Beringian lineage (Alsos *et al.* 2005, Eidesen *et al.* 2007). The Japanese archipelago, however, was omitted from these phylogeographic analyses (Fig. 2 in Eidesen *et al.* 2007). We have therefore attempted to add information by sampling cpDNA haplotypes of *V. uliginosum* sampled from plants in the Japanese archipelago.

Fresh leaves of *Vaccinium uliginosum* were collected from five Japanese mountain sites (Table 1). At each site, we sample six to eight plants separated by at least 20 m. Additionally, we obtained two herbarium specimens of *V. uliginosum* collected at a single site on the Kamchatka Peninsula. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The *trnL-trnF*

(Taberlet *et al.* 1991) and *trnS-trnG* spacers (Hamilton 1999) of the noncoding cpDNA regions were sequenced according to the method of Alsos *et al.* (2005). PCR amplification was performed using the "rpl16" cycling program of Shaw *et al.* (2005), and the PCR products were purified using 0.1 × ExoSAP (GE Healthcare) solutions and sequenced directly using 0.1 × diluted BigDye v.3.1 (Applied Biosystems). The forward and reverse strands of each cpDNA region were sequenced independently. Their sequences were determined using an ABI Prism 3130 automated sequencer (Applied Biosystems). We used *Vaccinium myrtillus* (sect. *Myrtillus*) and *Vaccinium vitis-idaea* (sect. *Vitis-idaea*) as outgroups based on information in Eidesen *et al.* (2007). Bayesian phylogenetic analysis was conducted using MrBayes v.3.1.1 (Ronquist & Huelsenbeck 2003), with the GTR+I model selected based on the value of Akaike's information criterion using MrModeltest v.2.2 (Nylander 2004). The analysis was run for 1,000,000 generations in a four-chain sampling tree every 100th generation. At the end of each run, we considered the sampling of the posterior distribution to be adequate if the average standard deviation of split frequencies was < 0.01. Trees prior to log likelihood stabilization and convergence (burn in = 2,500) were discarded before a majority rule consensus tree was generated. Posterior probabilities were estimated on 7,500 trees.

This analysis revealed three cpDNA haplo-

TABLE 1. Materials and their sources analyzed for cpDNA variation of *Vaccinium uliginosum*.

Locality	Coordination	No. of plants	cpDNA haplotype
Mt. Kisokomagatake, Central Honshu, Japan	35.78°N/137.81°E	6	J (6)
Mt. Chyogutake, Central Honshu, Japan	36.29°N/137.73°E	6	J (6)
Mt. Shirouma, Central Honshu, Japan	36.73°N/137.76°E	6	J(4), U(1), V(1)
Mt. Asama, Central Honshu, Japan	36.40°N/138.48°E	6	J (6)
Taisetsu Mountains, Hokkaido, Japan	43.55°N/142.87°E	8	J (8)
Esso, Central Kamchatka, Russia	55.97°N/158.68°E	2	J (2)

Haplotype J was previously identified by Alsos *et al.* (2005), whereas haplotype U and V were found in this study. Numbers in parentheses following haplotype represent number of individuals for each haplotype.

types (J, U, and V) at the five sites in the Japanese archipelago and one haplotype (J) at the site on the Kamchatka Peninsula (Table 1). Haplotype J, which was previously identified by Alsos *et al.* (2005), was detected at all six sites. Haplotypes U and V were newly detected on Mt. Shirouma, central Honshu, Japan. These haplotypes con-

tained one single-nucleotide polymorphism each compared to the haplotype J sequence, and were located in the *trnL-trnF* and *trnS-trnG* regions, respectively. Their sequences have been deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers AB570250 and AB570251.

Figure 1 presents a Bayesian phylogram of the

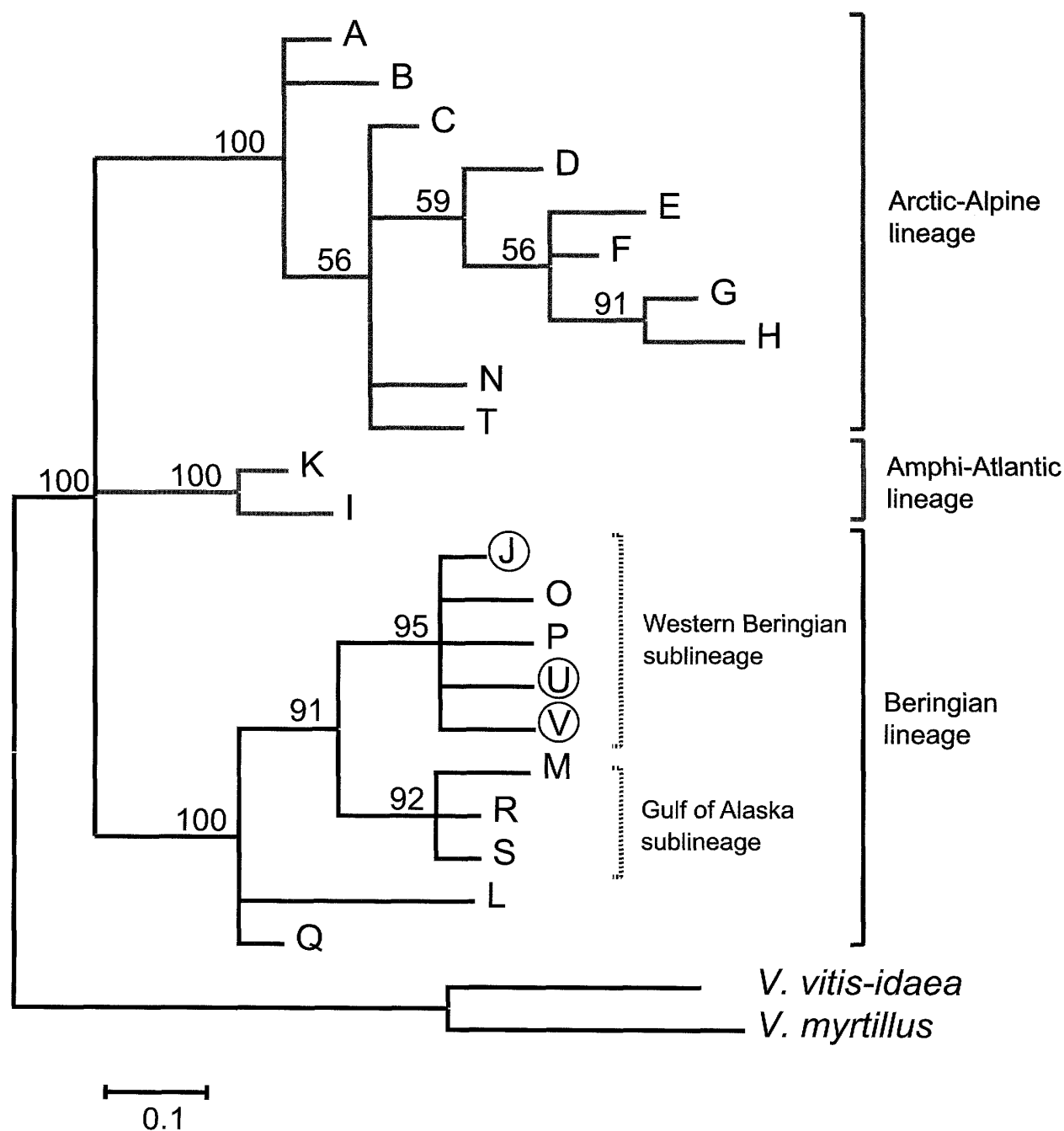


FIG. 1. Bayesian phylogram of the cpDNA haplotypes of the *trnL-trnF* and *trnS-trnG* intergenic spacer regions of *Vaccinium uliginosum*, which extends the previous datasets constructed by Alsos *et al.* (2005) and Eidesen *et al.* (2007). Circled letters denote the haplotypes detected in the Japanese archipelago and the Kamchatka Peninsula. Posterior probability values are shown above the branches.

22 haplotypes (A through V) that extends the previous datasets (Alsos *et al.* 2005, Eidesen *et al.* 2007). The tree was grouped into the Arctic-Alpine, Amphi-Atlantic, and Beringian lineages, as was demonstrated in previous studies. All three haplotypes detected in the Japanese archipelago and the Kamchatka Peninsula (J, U, and V) belonged to the Beringian lineage. Beringia, which encompasses the region from the Kolyma River in northeastern Russia to the Mackenzie River in Canada, is arguably the best-documented refugium for circumpolar plants, based on the hypothesis of Hultén (1937). During the last glacial period, Beringia remained unglaciated and so was able to sustain a tundra ecosystem. There is now excellent molecular, fossil, and phylogeographic evidence to support the Beringia refugium hypothesis for arctic and alpine plants (reviewed in Abbott & Brochmann 2003). Beringia also served as a land bridge between Eurasia and North America when sea levels fell by 100 to 135 m during the Quaternary period (Hopkins 1973). De-

Chaine (2008) inferred the distribution of the cp-DNA haplotypes of *V. uliginosum* to select a model of population divergence that reflected the importance of the Bering land bridge but not the Bering Sea dispersal barrier. Those findings suggest that *V. uliginosum* could have survived continuously throughout the Quaternary within Beringia, thereby providing a source of *V. uliginosum* for future colonization events.

Within the Beringian lineage, two sublineages were found: (i) the western Beringian lineage contains haplotypes from the Seward Peninsula in Alaska, the Chukchi Peninsula in northeastern Russia, the Kamchatka Peninsula, and the Japanese archipelago. The Gulf of Alaska sublineage (ii) is composed of haplotypes from the Aleutian Islands and south-central to southwestern Alaska (Figs. 1, 2). The geographical discrimination of the two sublineages indicates that the western Beringian sublineage includes populations that migrated to the Japanese archipelago. During glacial periods, several land bridges were formed

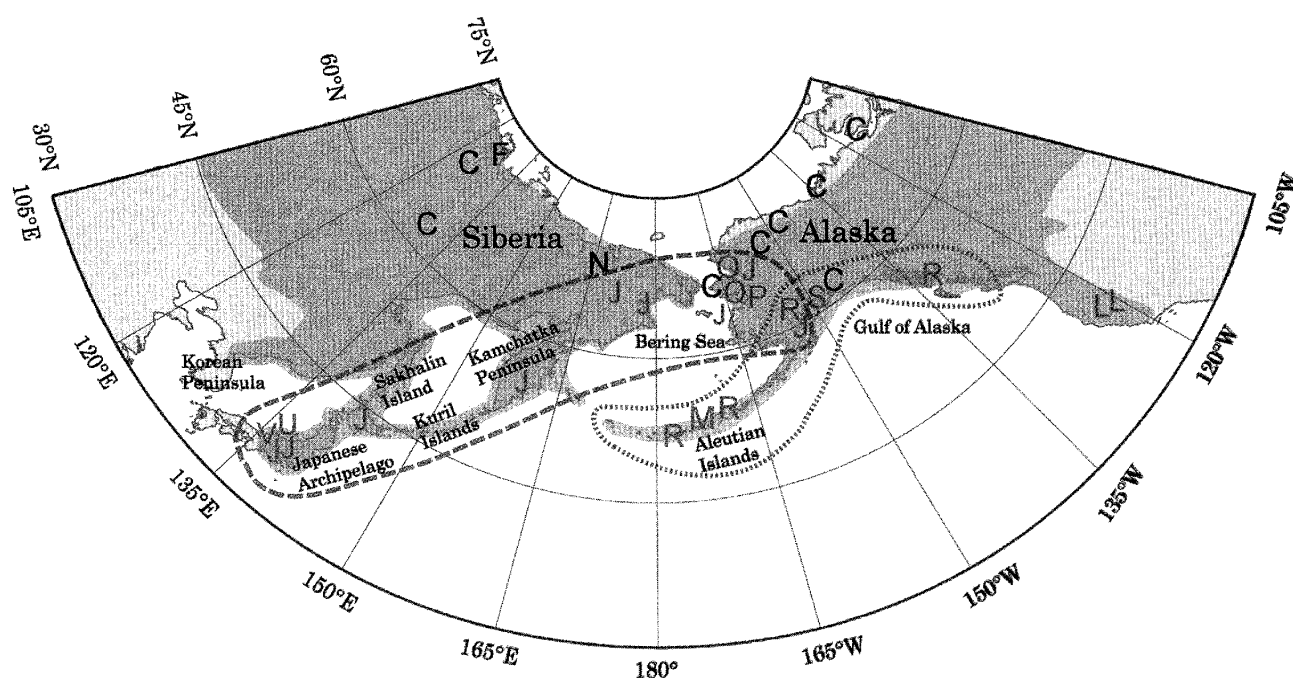


FIG. 2. Geographical distribution of the Beringian lineage of *Vaccinium uliginosum*, which extends the previous datasets constructed by Alsos *et al.* (2005) and Eidesen *et al.* (2007). Green letters, Beringian lineage; blue letters, Arctic-Alpine lineage. Dashed and dotted lines denote the western Beringia sublineage and the Gulf of Alaska sublineage, respectively (Fig. 1). The geographical distribution of the species (Hultén 1968) is shaded.

around an island chain along the East Asian littoral from Beringia to the Japanese archipelago (e.g., bridges in the Bering Strait, between the Aleutian Islands, between the Kuril Islands, in the Tartary Strait, in the La Pérouse Strait, and in the Tsugaru Strait; Ono & Igarashi 1991). The land bridges between Beringia and the Japanese archipelago were probably effective corridors for the migration of plants and animals. Although long distance seed dispersal of *V. uliginosum* by animals or birds could influence the current genetic structure, the migration scenario is the most parsimonious interpretation for the haplotype data. It should be noted, however, that uncertainty over the phylogeographic relationship of the populations of *V. uliginosum* on the Korean Peninsula and those in the Japanese archipelago must be resolved before we can determine whether a southern corridor that permitted migration through the Korean Peninsula was effective. A key observation of the present study is that Beringia appears to be the phylogeographic center of origin of the *V. uliginosum* populations in the Japanese archipelago. Further comparison of the phylogeography of several species will provide a more complete picture of how circumpolar plants achieved their current distribution in the Japanese archipelago.

As has been found for many arctic and alpine plants (reviewed in Brochmann *et al.* 2004), polyploidization events complicate the history of *V. uliginosum*. On a global scale, the Amphi-Atlantic lineage is composed of only tetraploids, whereas the Arctic-Alpine and Beringian lineages contain diploids, triploids, and tetraploids (Eidesen *et al.* 2007). Cytological observations revealed that plants of *V. uliginosum* from Mt. Asama, central Honshu, Japan, were hexaploid (Hara 1953). The existence of hexaploidy in the Japanese archipelago, which was not reported by Eidesen *et al.* (2007), suggests that the Beringian lineage is composed of multiple polyploids. Thus, additional cytological and molecular analyses to further clarify the evolutionary history of *V. uliginosum* in the Japanese archipelago are needed.

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